

Paper Chromatography of 2,4-Dinitrophenylhydrazones

Estimation of 2-Alkanone, *n*-Alkanal, Alk-2-enal, and Alk-2,4-dienal Derivatives

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► The application of paper chromatographic methods of separating 2-alkanone, *n*-alkanal, alk-2-enal, and alk-2,4-dienal 2,4-dinitrophenylhydrazones into individual compounds has been examined. In experiments with four mixtures the mean recoveries were consistent and showed small variations. In over-all recovery, average deviation from the mean was $\pm 3.4\%$. Recovery decreased from the 2-alkanones to the alk-2,4-dienals because of differences in stability of the classes. However, this was reflected in but small error in proportions of classes and the ratio of individual compounds found.

RECENT publications have described rapid paper chromatographic methods of separating mixtures of 2-alkanone, *n*-alkanal, alk-2-enal, and alk-2,4-dienal 2,4-dinitrophenylhydrazones into classes (2, 4, 5) and each class into individual compounds (1-4). These methods have been applied to the determination of changes in the proportions of steam-volatile monocarbonyl classes with the autoxidation of pork fat (5),

and to the identification of such compounds volatilized from a rancid pork fat (3). In the latter study, tentative quantitative data were reported for the classes and individual compounds. Aside from the inevitable mechanical losses in the manipulations, it was recognized that the most serious obstacle to quantitative application was the variation in stability of the different classes (2). The alk-2,4-dienal derivatives are particularly sensitive to light and air (2, 6). This work was undertaken to determine the quantitative capabilities of the methods.

EXPERIMENTAL

Solvents, reagents, materials, and equipment were the same as used in similar operations described in earlier papers (1-5).

Authentic monocarbonyl 2,4-dinitrophenylhydrazones employed have been described (1, 2). Stock solutions of each hydrazone in carbon tetrachloride were prepared containing the equivalent of approximately 25 mg. per liter (25 γ per ml.). Suitable volumes were taken from each stock solution to make up 100-ml. solutions of 30 μ moles per liter con-

centration. Aliquots of the various solutions were used to prepare the mixtures used in the experiments.

Separation into classes (2, 5) and resolution of the classes into individual compounds (1-3) were performed as described in earlier papers. Proportions of classes (2, 5) could be determined from spots extracted from three paper strips and measured spectrophotometrically in 3.00 ml. of carbon tetrachloride. However, it was necessary to accumulate a number of paper strips from the class separation to provide sufficient material for estimation of the individual compounds (3). Spectrophotometric measurements of absorbance were usually made at the wave length of maximum absorption in 3.00 ml. of carbon tetrachloride. However, it was necessary to measure the spectra of compounds separated on vaseline-impregnated paper in alcoholic alkali (3), because of the presence of a persistent impurity (1, 3) that absorbed at the lower wave lengths.

Every practical effort was made to protect the carbonyl derivatives from the effects of light and air. Spotted paper strips were chromatographed immediately, and separated spots extracted and measured as quickly as possible. Solvent was removed from

extracts on the steam bath with a jet of nitrogen.

RESULTS AND DISCUSSION

The standard solutions containing 3 μ moles of 2,4-dinitrophenylhydrazones per 100 ml. were found to have the following average absorbance values at their wave length of maximum absorption: 2-alkanones 0.615, *n*-alkanals 0.615, alk-2-enals 0.828, and alk-2,4-dienals 1.12. These values were used as the basis for calculation of results.

Mixture 1. Equal volumes of 2-alkanones C_4 to C_9 , C_{11} , and C_{13} , *n*-alkanals C_3 to C_{14} , alk-2-enals C_3 to C_{12} , and alk-2,4-dienals C_5 to C_{12} were combined. This mixture of 38 compounds contained 0.0789 μ mole of each compound in 100 ml. of solution. It included only those members of the four homologous series that separate in their proper classes. The solution had an absorbance value at λ_{max} . of 0.70. (The sum of calculated absorbance

values at λ_{max} . of the four classes was 0.778.) In the class separation, 2.15 ml. of the mixture (2, 3) were used to spot each paper strip. In the separation into individual compounds, 54 class separation paper strips were used for the 2-alkanones and *n*-alkanals, 42 strips for the alk-2-enals, and 36 strips for the alk-2,4-dienal class. Results were calculated on the basis of 100 ml. of mixture and are shown in Table I. In the class separation, the proportion of alk-2,4-dienals was somewhat low and those of the other classes were a little high, because of the much lower recovery of the alk-2,4-dienal class. In the separation into individual compounds, recovery was higher and more uniform. Possibly the sensitive compounds were protected by the impregnating agents. Proportions were not greatly changed by the separation into individual compounds. However, unsaturated classes were low and saturated classes high. This was reflected in over-all recovery which decreased with degree of un-

saturation. Average deviation from the mean in the over-all recovery was an acceptable $\pm 3.0\%$.

Mixture 2. This mixture was made up of equal volumes of 2-alkanones C_3 to C_9 , C_{11} , and C_{13} , *n*-alkanals C_1 to C_{14} , alk-2-enals C_3 to C_{12} , and alk-2,4-dienals C_5 to C_{12} . The resulting solution contained 41 compounds of equal concentration (3.00 μ moles per 100 ml.). This mixture differed from Mixture 1 because 2-alkanone C_3 separates in the *n*-alkanal class, and *n*-alkanals C_1 and C_2 separate with the alk-2-enal class (2). In separation into individual compounds, this results in inseparable mixtures of 2-alkanone C_3 with *n*-alkanal C_3 and *n*-alkanal C_2 with alk-2-enal C_3 . It was necessary to study this separation to determine the influence of the inseparable groups on quantitative determinations. The resulting data are shown in Table II for 100 ml. of mixture. Results were very similar to those obtained with Mixture 1 for the separable compounds.

Table I. Analysis of 2,4-Dinitrophenylhydrazone Mixture 1

	Class Separation							Individual Separation				Over-all Recovery	
	% Based on Absorbance at λ_{max} .		μ mole		% recovery	Proportions, μ mole %		μ mole		% proportions found	% recovery	% Av. dev. from mean	
	Pres-ent	Found	Pres-ent	Found		Pres-ent	Found	Found	Av. dev. from mean				
2-Alkanones, C ₇ -C ₉ , C ₁₁ , C ₁₃	16.65	17.68	0.632	0.53	84.0	21.0	22.0	0.47	±0.0018	87.9	22.7	73.9	±2.3
<i>n</i> -Alkanals, C ₃ -C ₁₄	24.98	25.43	0.947	0.77	81.3	31.6	31.9	0.67	±0.0017	87.5	32.4	71.2	±3.2
Alk-2-enals, C ₃ -C ₁₂	28.03	29.04	0.789	0.65	82.4	26.4	27.0	0.53	±0.0026	81.1	25.6	66.8	±3.3
Alk-2,4-dienals, C ₅ -C ₁₂	30.34	27.85	0.632	0.46	72.9	21.0	19.1	0.40	±0.0026	86.5	19.3	63.1	±3.2
Total			3.000	2.41				2.07					
Average					80.2				±0.0022	85.6		68.8	±3.0

Table II. Analysis of 2,4-Dinitrophenylhydrazone Mixture 2

	Class Separation							Individual Separation				μ mole Over-all			
	μ mole Present Found recovery			% Proportions As Separation Goes			μ mole		% recovery	% proportions	% recovery	% av. dev. from mean			
				Actual present	Pres- ent Found		Total found	Av. dev. from mean							
2-Alkanones, C ₄ -C ₉ , C ₁₁ , C ₁₃		0.586	0.51	87.0	21.9	19.6	21.5	0.43	± 0.003	84.3	21.2	73.3	± 4.7		
2-Alkanone C ₃	0.0732	0.951	0.77	80.9				31.6	32.5	0.09	± 0.002	87.0	33.2	61.5	± 2.8
n-Alkanal C ₃	0.0732														
n-Alkanals C ₄ -C ₁₄	0.805														
n-Alkanal C ₁	0.0732	0.877	0.69	78.6	34.1	29.2	29.1	0.58	0.04	82.6	28.2	72.0	68.1		
n-Alkanal C ₂	0.0732							0.10				54.6			
Alk-2-enal C ₃	0.0732							0.43				± 0.003		65.4	
Alk-2-enals C ₄ -C ₁₂	0.658	0.586	0.40	68.3	19.6	19.6	16.9	0.43	± 0.003	87.5	17.4	59.7	± 4.3		
Alk-2,4-dienals C ₅ -C ₁₂								0.35	± 0.003	85.4	64.9	± 4.1			
Total		3.00	2.37					2.02							
Average				78.7					± 0.003	85.4		64.9	± 4.1		

Table III. Analysis of 2,4-Dinitrophenylhydrazone Mixture 3

	Stand- ard Solu- tions Max. Absorb- ance	Composition Mixture, μmoles		Class Separation μmole					Individual Separation				Over-all μmole	
				Found	% Re- cov- ery	Actual classes	Proportions, %		Indi- vidual μmoles	% re- cov- ery	Proportions			
		As Separation Goes					% pres- ent	% found						
		Indi- vidual com- pounds	As classes								Pres- ent	Found	% pres- ent	% found
<i>n</i> -Alkanals														
C ₅	1.23	0.060	1.756	1.39	79.2	81.8	79.8	80.3	0.039	83.2	2.73	2.71	67.2	±1.9
C ₆	34.29	1.673							1.113		76.05	77.35		
C ₉	0.47	0.023							0.015		1.05	1.04		
C ₁₁	0.26	0.013							0.009		0.59	0.63		
			0.043						0.021		1.36	1.46		
Alk-2-enals														
C ₇	0.62	0.030	0.216	0.20	77.2	9.8	11.8	11.6	0.032	81.5	2.23	2.22	62.0	±5.0
C ₈	1.35	0.049							0.026		1.95	1.81		
C ₉	1.18	0.043							0.030		2.05	2.08		
C ₁₀	1.04	0.045							0.025		2.23	1.74		
C ₁₁	1.35	0.049							0.020		1.36	1.39		
	0.83	0.030												
Alk-2,4-dienals														
C ₇	0.23	0.006	0.185	0.14	75.7	8.4	8.4	8.1	0.004	77.9	0.27	0.28	60.9	±3.8
C ₈	0.28	0.008							0.005		0.36	0.35		
C ₁₀	6.04	0.162							0.095		7.36	6.60		
C ₁₁	0.35	0.009							0.005		0.41	0.35		
Total Average			2.20	1.73	77.4				1.439	80.9			63.4	±3.9

Table IV. Analysis of 2,4-Dinitrophenylhydrazone Mixture 4

	Standard Solutions Max. Absorbance	Composition Mixture		Class Separation				Individual Separation				Over-all μ mole	
				Found, μ mole	%	Proportions		Found, μ mole	%	Proportions		%	% av. dev. from mean
		% present	% found			% present	% found			% recovery			
<i>n</i> -Alkanals													
C ₅	11.50	1.122	1.199	0.97	80.9	42.8	44.5	0.784	85.9	40.04	43.45	66.1	± 2.9
C ₈	0.31	0.030						0.020		1.07	1.11		
C ₉	0.48	0.047						0.029		1.68	1.60		
Alk-2-enals													
C ₇	2.56	0.186	0.859	0.67	78.0	30.7	30.7	0.113	84.0	6.63	6.25	65.7	± 2.6
C ₈	2.21	0.160						0.114		5.71	6.30		
C ₉	2.56	0.186						0.123		6.63	6.79		
C ₁₀	2.32	0.167						0.107		5.96	5.91		
C ₁₁	2.21	0.160						0.106		5.71	5.86		
Alk-2,4-dienals													
C ₇	0.25	0.013	0.744	0.54	72.6	26.5	24.8	0.007	76.5	0.46	0.39	53.1	± 2.1
C ₁₀	13.48	0.723						0.402		25.80	22.22		
C ₁₁	0.15	0.008						0.004		0.31	0.12		
Total Average			2.802	2.18	77.2			1.809	82.1			61.6	± 2.5

Table V. Survey of Recoveries

	Class Separation Recovery, %				Individual Separation Recovery, %					Over-all Recovery, %		
	2-Alka-nones	<i>n</i> -Alka-nones	Alka-2-enals	Alka-2,4-dienals	Total mean	2-Alka-nones	<i>n</i> -Alka-nones	<i>n</i> -Alka-enals	Alka-2,4-dienals	Total mean	Total mean	Av. dev. from mean
Mixture												
1	84.0	81.3	82.4	72.9	80.2	87.9	87.5	81.1	86.5	85.6	68.8	± 3.0
2	87.0	80.9	78.6	68.3	78.7	84.3	87.0	82.6	87.5	85.4	64.9	± 4.1
3	...	79.2	77.2	75.7	77.4	...	83.2	81.5	77.9	80.9	63.4	± 3.9
4	...	80.9	78.0	72.6	77.2	...	85.9	84.0	76.5	82.1	61.6	± 2.5
Mean	85.5	80.6	76.6	72.4	78.4	86.1	85.9	82.3	82.1	83.5	64.7	...
Av. dev.	± 1.5	± 0.7	± 2.9	± 2.0	± 1.1	± 1.8	± 1.4	± 1.0	± 4.9	± 2.0	± 2.2	± 3.4
Diff. between mean class recovery and total recovery	+7.1	+2.2	-1.8	-6.0	...	± 2.6	+2.4	-1.2	-1.4

Mixture 3. This mixture was composed of the kinds and relative amounts of monocarbonyls identified in the steam distillate of an unheated rancid pork fat (3). Table III shows the absorbance values of the standard solutions used in preparing the mixture. The solution analyzed was made up by combining 1.00 ml. of each of the standard solutions. Data represent amounts found in 14.00 ml. of mixture. In general, results were similar to those found in the preceding experiments but recoveries were much more uniform. There was good agreement between the proportions of individual compounds present and those found.

Mixture 4. This mixture was composed of the proportions of steam-volatile monocarbonyls found (3) in the rancid pork fat after heating at 165° C. Two milliliters of each of the standard solutions shown in Table IV were used to make 22.00 ml. of mixture. Quantities shown in the data are those found for that volume of mixture. Recoveries showed the same trend observed in the previous experiments. Again there was good agreement between the proportions

of individual compounds present and those found. However, proportions were high for the saturated, and low for the alk-2,4-dienal class.

Table V surveys the results of the four experiments. Mean recoveries in the class separation, individual separation, and over-all were consistent and showed small variations. Mean recoveries for the classes in the two separations were also consistent and had low deviations. In the class separation, there were very definite differences in recovery between the classes. Recovery decreased from the 2-alkanones to the alk-2,4-dienals. This was due to differences in stability of the classes, and is the chief cause of error in the method. There was much less difference in class recoveries in the separation into individual compounds. It is probable that chromatography on the impregnated paper gives some degree of protection to the sensitive classes. Results indicate that the method is flexible and consistent, and possesses good reproducibility and precision.

The paper chromatographic methods require only simple equipment (1, 2).

Class separation requires 1 1/4 hours (2), and the separation into individual compounds (1) 1 3/4 hours for low molecular weight compounds and 6 hours for the derivatives of higher molecular weight. Time of analysis is naturally influenced by the proportions of compounds present, but generally a complete analysis can be made within 3 days.

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